clear, however, whether the latter associations are independent of the main association of DRB1*07:01 or whether they are secondary and result from linkage disequilibrium with this allele of DRB1. Nonetheless, it is worth noting that GWAS also revealed a second HLA susceptibility factor whose association appears to be independent of the major HLA class II association (Jin *et al.*, 2010; Quan *et al.*, 2010).

A next step will be to identify the self-antigen binding the DRB1*07:01 molecule, leading to autoimmune destruction of melanocytes. GWAS has revealed associations with alleles of tyrosinase (TYR), which encodes the protein that catalyzes melanin biosynthesis (Jin *et al.*, 2010), and this might be considered a vitiligo antigen. Other proposed prime targets of the immune response in vitiligo include human melanoma antigen recognized by T-cells (MART-1), gp100/the silver homolog (SILV), tyrosinase-related protein 1 precursor (TYRP1), and tyrosinase-related-protein-2 (TYRP2) (Le Poole and Luiten, 2008).

The identification of HLA factors, whose role in antigen presentation is central and clear in the association of immune responses with susceptibility to vitiligo, allows one to postulate an autoimmune phenomenon in the cause of this disease. Improved understanding of its altered pathway is one more step toward the development of specific and efficient therapies for this devastating disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Control of Central and Peripheral Tolerance to Melanocyte Differentiation Antigens by GILT

Jürgen C. Becker[1] and David Schrama[1]

The strict control of the T-cell receptor repertoire is essential for prevention of autoimmune diseases. The repertoire of T cells is primarily formed in the thymus through positive and negative selection. The risk of an incomplete removal of autoreactive T cells necessitates additional means to maintain peripheral tolerance. There is increasing evidence that the interferon (IFN)-γ-inducible lysosomal thiol reductase (GILT) allows peripheral tolerance to a melanocyte differentiation antigen by induction of specific regulatory T cells.

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A functionally competent immune system is essential to the health of higher developed organisms. T cells play a major role in guiding the immune system to recognize and remove specific infectious agents and neoplastic cells, while also tolerating self-antigens to avoid autoimmunity (Andersen *et al.*,

[1]*General Dermatology, Medical University of Graz, Graz, Austria*

Correspondence: Jürgen C. Becker, General Dermatology, Medical University of Graz, Auenbruggerplatz 8, A-8036 Graz, Austria. E-mail: juergen.becker@medunigraz.at

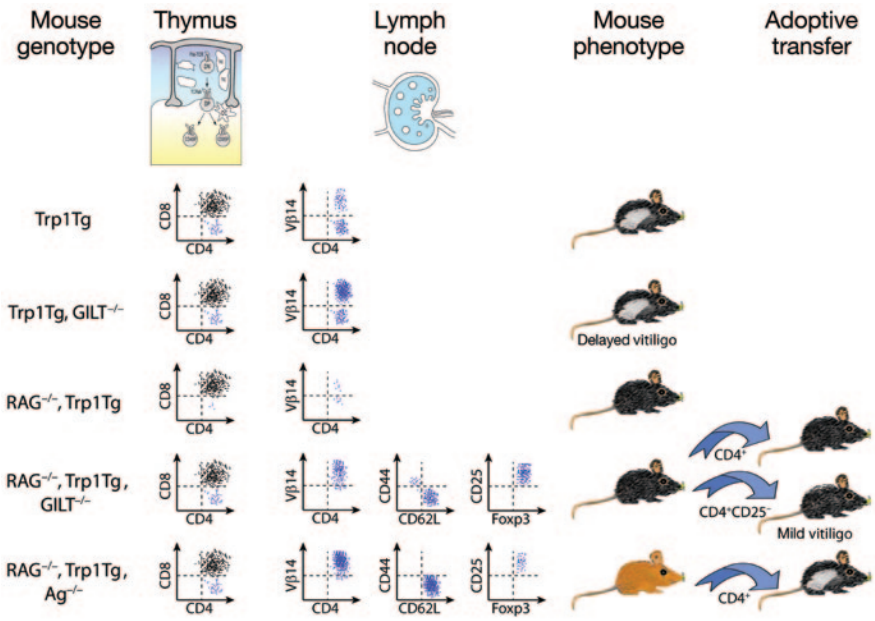

Figure 1. Effect of GILT on central and peripheral tolerance control. In Trp1 transgenic mice, Trp1-specific T cells are probably rescued by endogenous T-cell receptors from thymic depletion. Consequently, those Vβ14Vα3.2 can be detected in the periphery and cause vitiligo. In the absence of IFN-γ–inducible lysosomal thiol reductase (GILT), vitiligo is delayed even though more transgenic T cells are present in the periphery. In RAG$^{-/-}$ Trp1 transgenic mice, the thymus lacks CD4 single-positive cells almost completely; consequently, transgenic T cells are hardly present in the periphery, and these mice do not develop vitiligo. In the absence of GILT, however, the mice do have both CD4 single-positive cells in the thymus and transgenic T cells in the periphery. The latter display partially the CD44^{+}CD62l^{-} effector memory phenotype. Mice not expressing Trp1 (Ag$^{-/-}$) similarly escape negative selection in the thymus and have even larger number of transgenic T cells in the periphery, but these are largely of a naive phenotype. In addition, the frequency of regulatory T cells (CD4^{+}Vb14^{+}Foxp3^{+}CD25^{+}) is fourfold greater in GILT$^{-/-}$ mice compared with that observed in Ag$^{-/-}$ mice. Upon adoptive transfer of CD4^{+} cells, only those obtained from the Ag$^{-/-}$ mice cause vitiligo in the recipient animals. However, transfer of only CD4^{+}CD25^{-} cells from the GILT$^{-/-}$ mice causes mild vitiligo.

2006). The ability of T cells to recognize a wide variety of foreign antigens is a result of random recombination events that generate T-cell receptors (TCR). However, because of the stochastic assembly of the TCR, T cells are likely to recognize both self- and foreign antigens. On the one hand, the wide range of foreign antigen specificities generated ensures a nearly unlimited potential to recognize pathogens; on the other hand, the production of self-reactive T cells can result in debilitating autoimmunity. Thus, a strict quality control of developing T cells is essential to prevent autoimmune diseases. To this end, the repertoire of T cells is formed primarily in the thymus through positive and negative selection of developing thymocytes (Derbinski and Kyewski, 2010). Central tolerance is established within the thymus by purging self-reactive thymocytes, reducing the propensity for autoreactivity among mature T cells after they gain entry to the periphery.

The fact that the recognition of self-antigens not only is essential for thymocytes' survival and lineage commitment but also may induce cell death remains incompletely understood. Moreover, the possibility—or risk—of incomplete removal of T cells with self-antigen specificity necessitates additional mechanisms to maintain peripheral tolerance, especially when central tolerance inductions fail (Metzger and Anderson, 2011). These include the additional mechanisms of clonal deletion of mature autoreactive T cells in the periphery and active suppression of their activation by regulatory T cells (Tregs).

In this issue, Rausch and Hastings report findings on the role of interferon (IFN)-γ-inducible lysosomal thiol reductase (GILT) for CD4 T-cell tolerance to a melanocyte differentiation antigen, adding substantially to our understanding of central and peripheral tolerance to self-antigens (Rausch and Hastings, 2012).

Antigen processing and presentation via the class II pathway (Figure 1) are important for successful CD4^{+} T-cell stimulation and the development of the CD4^{+} T-cell repertoire. It has been reported that intracellular proteases are regulated by GILT (Goldstein *et al.*, 2008). Intracellular proteases such as cysteinyl and aspartyl cathepsins (e.g., Cat B, D, or S) are essential for the degradation of endogenous and exogenous antigens, and each performs an important role in the processing of peptides for presentation. Peptides cannot be loaded into the class II binding groove until CLIP is removed by the nonclassic molecule HLA-DM; after subsequent insertion of a high-affinity peptide, stable human leukocyte antigen class II–peptide complexes are presented on the cell surface for CD4^{+} T-cell recognition. Indeed, GILT colocalizes with Cat B and D, thereby indicating that GILT is crucial for antigen processing and presentation via the class II pathway (Goldstein *et al.*, 2008). Moreover, the reduction of disulfides is important in the processing of tumor antigens such as tyrosinase-related protein 1 (TRP-1), tyrosinase, gp-100, Mart-1, and NY-ESO-1, all of which contain large numbers of cysteine residues (Rausch *et al.*, 2010).

Based on this role of GILT for antigen processing and presentation and the relevance of MHC class II–restricted antigen presentation for the development of the T-cell repertoire, Rausch and Hastings (2012) hypothesized that GILT has the potential to shape CD4^{+} T-cell tolerance to self-antigens. To test this hypothesis, they took advantage of a class II–restricted TRP1-specific TCR transgenic RAG1$^{-/-}$ mouse strain that was crossed with GILT$^{-/-}$ or white-based brown (TRP1Bw) mice; the latter contains a radiation-induced inversion interrupting the gene encoding TRP1 (Muranski *et al.*, 2008). These experiments revealed an additional role for GILT in the maintenance of tolerance to TRP1. As expected, TRP1-specific thymocytes were deleted

centrally in the presence of GILT and TRP1. By contrast, CD4 single-positive thymocytes and peripheral T cells developed in the absence of GILT or TRP1 antigen, demonstrating that GILT is required for negative selection of TRP1–specific thymocytes. Surprisingly, however, neither GILT-expressing nor GILT$^{-/-}$ TRP1-specific TCR transgenic mice developed vitiligo.

> GILT has the potential to shape CD4$^+$ T-cell tolerance to self-antigens.

Thus, although TRP1-specific T cells escape thymic deletion in the absence of GILT, they are nevertheless tolerant to TRP1, suggesting the presence of effective mechanisms of peripheral tolerance. Indeed, TRP1-specific T cells developing in the absence of GILT are characterized by an impaired capacity to produce IL-2 and IFN-γ upon specific or nonspecific stimulation. This concept was explained by the fourfold increase in TRP1-specific Tregs in GILT-deficient mice compared with that of TRP1-deficient animals. Indeed, depletion of Tregs partially restored the ability of GILT-deficient TRP1-specific CD4$^+$ T cells to induce vitiligo when transferred to RAG$^{-/-}$ mice.

Tregs are critical in maintaining self-tolerance and immune homeostasis, and their development and function depend on the expression of the transcription factor FoxP3. CD4$^+$FoxP3$^+$ T cells are functionally and phenotypically heterogeneous, providing plasticity to immune activation and regulation. Deletion of or mutations in the FOXP3 gene lead to severe autoimmune disease in both humans and mice caused by alteration or disruption of Treg function.

The exact process of Treg selection is not fully understood, but it appears to be determined by the affinity of the interaction with the peptide–major histocompatibility complex (MHC) complex: a T cell that receives strong signals will undergo apoptotic death; a cell that receives weak signals will survive and be selected to become an effector cell (Moon *et al.*, 2011; Moran *et al.*, 2011). If a T cell receives an intermediate signal, then it will become a Treg. In addition, the overall avidity of the interaction between self-reactive thymocytes and their cognate self-peptide–MHC complexes may also direct T-cell fate. In the model system used by Rausch and Hastings (2012), the number of TRP1 peptide–MHC complexes is probably reduced in the absence of GILT. Therefore, the absence of GILT reduces the avidity of TCR interaction with peptide–MHC complexes and shifts the fate of TRP1-specific thymocytes from central deletion to Treg development.

Rausch and Hastings (2012) demonstrate that GILT plays a critical role in regulating peripheral CD4$^+$ T-cell tolerance to an endogenous skin-restricted antigen relevant to controlling autoimmunity. However, additional work is required to expand our knowledge of GILT and its function during the induction and maintenance of peripheral tolerance. The results of such studies may contribute to answering the question of whether GILT's striking ability to control immune responses to melanocyte differentiation antigens might be harnessed for melanoma therapy—a notion that has already been explored *in vitro* and is supported by *in vivo* observations in patients with melanoma (Goldstein *et al.*, 2008; Becker *et al.*, 1999; Delfino *et al.*, 2011).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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